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PRINCIPAL INVESTIGATOR: Doctor Jacob W. Petrich

CONTRACTING ORGANIZATION: Iowa State University of Science and Technology
Ames, Iowa 50011

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6. AUTHOR(S)
Doctor Jacob W. Petrich

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Iowa State University of Science and Technology
Ames, Iowa 50011

E-Mail: jwp@iastate.edu

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Fluorescence spectra of scrapie-infected sheep eyes have been compared with those of healthy cow eyes. All of the eye parts have been investigated in detail and assessed for their ability to determine whether they provide a probe of TSE infection.

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Introduction

"Mad cow disease," in cattle, "chronic wasting disease," in deer and elk, scrapie in sheep, and Creutzfeldt-Jakob disease (CJD) in humans are examples of diseases known as transmissible spongiform encephalopathies (TSEs). TSEs are slowly progressive, insidiously degenerative diseases that affect the central nervous system (CNS) and that are usually accompanied by the production of "spongiform" changes in the brain. The resulting neurodegeneration causes a variety of cognitive, behavioral, and physical abnormalities. TSEs are believed to be transmitted by abnormal proteins, which are resistant to enzymatic degradation, called prions. Prions accumulate in the brain, spinal cord, and eye—that is, in central nervous system tissue. There is a granular substance known as lipofuscin that also accumulates in the central nervous system (neurons and in the eye). This substance, which is also undegradable and which originates from the presence of oxidants in cells, has been exploited by histologists and pathologists as a marker of age but remains largely uncharacterized. Lipofuscin is a highly fluorescent substance: when excited with light of a given color, it emits light of a redder color. An increase in lipofuscin accumulation is known to occur in CJD victims and in cases of experimental TSEs. Our own preliminary data indicate that the fluorescence spectra (the range of colors emitted after absorption of light) of scrapie-infected sheep brain is substantially different from that of noninfected sheep brain. We propose to isolate the substances comprised in lipofuscin in the CNS, especially the eye, and to characterize them with respect to their ability to absorb and emit light with the aim of exploiting this fluorescent property to assay for the presence of CNS tissue and for the presence of TSEs. We propose to establish a correlation between the intensity and the color of lipofuscin fluorescence and TSE infection, initially beginning with mice and hamsters, but also using sheep. We shall subsequently design a methodology using sensitive light-detection techniques to not only detect TSE-infected tissue in real time but to assay animals (and in principle humans) for TSEs by administering an ophthalmological-like examination. This examination would be rapid and harmless to the subject. The relevance and the impact of this proposed research are that it may provide a real-time, noninvasive technology, for detecting TSE-infected tissue and, more importantly, TSE related diseases in living animals. Although other methods are under development, no such real-time technology exists.

Body

Statement of Work: Development of Methods for the Real-Time and Rapid Identification and Detection of TSE in Living Animals Using Fluorescence Spectroscopy

Task 1.

To obtain spectroscopic data from a large statistical sampling of age-matched eyes of healthy and infected animals (mice, hamsters, sheep) in order to verify the hypothesis that TSEs may be detected by fluorescence spectroscopy. (The feasibility of this is already suggested by the data presented in Figure 5.) Months 1-12:

a. Obtain statistically significant samples of age-matched healthy and diseased eyes. Because lipofuscin accumulates with age, it is important to distinguish spectroscopic

differences arising from age differences from those arising from TSE infection. The limiting step for this Task is the time required "to age" the subjects. All the milestones may be accomplished concurrently. Months 1-12.

No progress has yet been made in this horribly crucial part of the project because our NADC collaborators have not yet received the funding to acquire the necessary control animals.

b. Submit the aqueous humor, vitreous humor, lens, retina, and optic nerve to spectroscopic examination by means of steady-state fluorescence and excitation spectroscopy in order to determine whether lipofuscin fluorescence is diagnostic for TSEs. Months 1-12.

Considerable progress has been made in this regard with sheep eyes and cow eyes. All the components of the eye have been dissected and subjected to fluorescence spectrometry. The most promising parts of the eye seem to be the cornea, lens, retina, and especially and not surprising, the optic nerve.

c. In so doing, determine which part of the eye, if any, is more susceptible to yielding information on TSE infection. Months 1-12.

The optic nerve is very promising in this regard

d. Verify that no other pigments in the eye obfuscate the fluorescent signature arising from the TSEs. Months 1-12.

Depending on the configuration of a real time device to scan the eye, the tapetum may contribute to significant light scattering.

Task 2.

To perform an exhaustive study of the fluorescence excitation and emission spectra of solid samples and extracts from diseased and healthy eyes in order to determine the most sensitive and most reliable spectral region to exploit for probing CNS tissue. Months 1-18:

a. Characterize the fluorescence quantum yield of the lipofuscin pigment extracted from the various parts of the eye. This will require establishing a protocol that successfully removes all the fluorescent pigments from the tissue. The goal is to quantify the number of fluorescent photons that one might expect to detect per 100 incident photons and consequently begin to obtain ideas of the requisite sensitivity of the device that is the ultimate subject of Task 3. In other words, taken as a whole, this information will determine the smallest amount lipofuscin that can be monitored using a given detector and a given excitation wavelength and intensity. Months 1-12.

b. Compare the fluorescence quantum yield of the isolated pigments with that of the tissue from the eye. Months 12-18.

To date, because of the lack of control systems to focus our study, all we are in a position to say is that the optic nerve provides by far the most intense fluorescent signal. We hope that in the near future our results will be more quantitative.

Task 3.

To design a prototype device to detect fluorescence from an eye *in vivo*, based upon the spectroscopic evidence accumulated. Months 18-36.

a. Depending on the results obtained from Tasks 1 and 2, we shall begin with either a green (532-nm) or blue (441-nm) laser source (both available in our laboratories). It is hoped ultimately that laser excitation will not be required because of the expense in the construction of a commercial instrument. We begin with these sources, however, in order to determine the minimum detection threshold that is required to perform a real-time investigation. It is important that the excitation intensities employed not produce damage to the eye of the subject, and these levels shall be carefully monitored. Months 18-24.

b. These results shall provide sensitivity guidelines. Detection limits will be determined, and possible signals that may interfere will be evaluated. In order to perform a real-time measurement, an optical signal should be detected in 100-300 milliseconds. Months 24-26.

c. Once these criteria have been met with the best instrumentation available to us (lasers, photomultipliers, CCDs), we shall scale down the technology to provide the most economical solution to the problem. Months 26-36.

Our results are still too preliminary to begin work on an actual device. Hopefully we can begin this part in the next year.

These tasks will involve, per year, 100 mice, 50 hamsters, and 20 sheep. Half of the subjects will be healthy; half will be infected. It is important that comparisons be made between age-matched healthy and infected subjects, in order to account properly for lipofuscin accumulation with age.

Key Research Accomplishments

- Establishment of a protocol for dissecting the animal eye safely in a controlled environment and decontaminating and disposing of waste materials.
- Acquisition of the fluorescence spectra of every part of the sheep eye.
- Determination that the optic nerve provides the most intense and structured fluorescence signal.

Reportable Outcomes

- A significant catalog of the optical spectra of the eyes of sheep and cow. Unfortunately, because we do not have age-matched spectra, these spectra are merely suggestive and cannot yet be considered publishable.

Conclusions

The acquisition of age-matched material (infected and noninfected) is crucial to the completion of this project and the transmission of the funding to the NADC colleagues is requested to be expedited. The optic nerve provides a very structured and rich spectrum. Tenuous comparisons of scrapie infected sheep eyes with those of healthy cow eyes indicate that the optic nerve may be the region upon which we might focus our attentions.

References

None to augment the body of the proposal.